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Phaeonectriella alba sp. nov. from the River Nile, Egypt

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ABSTRACT— A new species, *Phaeonectriella alba*, collected from the River Nile, Aswan Governorate, Egypt, is described and illustrated. Phylogenetic analyses of LSU rDNA sequences placed the new species with *P. lignicola* (the type species) in a highly supported clade. *Phaeonectriella alba* is characterized by conical, superficial, hyaline, membranous ascomata; 4- or 8-spored asci that are persistent, clavate, with a truncate apical thickening and a simple pore and cytoplasmic retraction below apex; early deliquescing catenophyses; and ascospores that are ellipsoid to broad ellipsoid, thin-walled, 1–3 septate, hyaline when young, becoming brown at maturity, with an apical germ pore in each cell and without appendages.

KEY WORDS—freshwater fungi, *Halosphaeriaceae*, *Microascales*, Middle East, subtropical fungi

Introduction

During an ongoing study of freshwater fungi colonizing herbaceous and woody material submerged in water around four islands in the southern part of the River Nile, Egypt, an undescribed species of *Phaeonectriella* R.A. Eaton & E.B.G. Jones was discovered. The genus was established to accommodate *P. lignicola* R.A. Eaton & E.B.G. Jones that was described from wooden blocks placed for 54 weeks in a cooling tower in Wales (Eaton & Jones 1970). *Phaeonectriella lignicola* was later recorded from test blocks exposed in water

[†] We regret to announce the sudden death of Prof. Ahmed M. Abdel-Raheem during the course of this work.

cooling towers in India (Natarajan & Udaiyan 1978) and from freshwater habitats in Taiwan (Jones 1995). Ascospore appendages were not mentioned in the original description of *P. lignicola* or the collection from Taiwan (Eaton & Jones 1970, Jones 1995), but Hyde & al. (1999) later identified *P. lignicola* from Mauritius that differed by having polar appendages and lacking polar germ pores. Hyde & al. (1999) also described a second species, *P. appendiculata* K.D. Hyde & al. from submerged wood in freshwater habitats in Australia and Philippines, with polar appendages and larger-sized ascospores. Jones (1995) assigned *P. lignicola* to *Halosphaeriaceae* after observing typical halosphaeriaceous pseudoparenchyma in the centrum that form catenophyses, and asci with an apical pore in the isolate from Taiwan. No molecular data are available for *P. appendiculata* or for the Mauritius specimen of *P. lignicola*.

Phaeonectriella has common morphological characters with the genus *Aniptodera* Shearer & M.A. Mill., including: hyaline ascomata; persistent asci with a truncate apical thickening and a simple pore and cytoplasmic retraction below apex; and ascospores with or without polar appendages. *Phaeonectriella* has thin-walled ascospores that become brown at maturity, with an apical germ pore and with or without polar appendages. In contrast, *Aniptodera* sensu stricto species have thick-walled ascospores that are hyaline and with or without polar appendages (Eaton & Jones 1970, Shearer & Miller 1977, Shearer & Crane 1980, Koch 1982, Shearer 1989). Another morphologically related genus is *Halosarpheia* Kohlm. & E. Kohlm. which has dark-colored ascomata; thin-walled asci that deliquesce early; and bi-celled, ellipsoidal, hyaline, thin-walled ascospores with polar unfurling appendages (Kohlmeyer & Kohlmeyer 1977). Molecular studies of the ribosomal genes suggested that *Aniptodera* and *Halosarpheia* are polyphyletic, and several new genera were named to accommodate phylogenetically distant taxa from the type species (Campbell & al. 2003; Pang & al. 2003; Jones & al. 2015, 2017). *Phaeonectriella lignicola* was placed in a distant clade from the type species of the two genera: *Aniptodera chesapeakeensis* Shearer & M.A. Mill. and *Halosarpheia fibrosa* Kohlm. & E. Kohlm. (Pang & al. 2003, this study).

Materials & methods

Sample collection

Submerged decayed culms of *Phragmites australis* (Cav.) Trin. ex Steud. (*Poaceae*) were collected from the River Nile in Aswan Governorate. Collected samples were placed in clean plastic bags and returned to laboratory. Samples were examined under

a stereomicroscope upon return to the laboratory, placed in sterile humid plastic boxes for incubation, and examined periodically over three months. Ascomatal squash of the new species was mounted in freshwater for all measurements and photography. Photographs were taken using an Olympus BX51 differential interference contrast light microscope and Optika view version 7.3.1.7 digital imaging system. Vertical sections of ascomata were prepared using Leica CM1100 cryostat. Herbarium materials of the new species were prepared by drying natural wood bearing the fungus at 60°C for 24 h. The herbarium material of the new species was deposited at Assiut University Mycological Centre, Assiut, Egypt (AUMC). An identifier number was registered (Mycobank 2017). Ascospores of the new species did not grow in culture.

DNA extraction, sequencing, and phylogenetic analysis

DNA was extracted directly from fruiting structures by collecting the ascomata from the surface of the natural wood carefully using clean, sterilized fine forceps. Ascomata were washed twice in sterile distilled water by centrifugation at 10000 rpm for 1 minute. Microbial DNA Extraction Kit was used for DNA extraction according to the manufacturer's instructions. Partial nuclear LSU rDNA sequence was amplified with the primers LROR and LR7 (Vilgalys & Hester 1990, Bunyard & al. 1994). Our attempts to obtain ITS and SSU rDNA sequences of the new species were unsuccessful. PCR reactions were carried out by Macrogen Inc., Korea using MGMTM Taq-HF DNA polymerase. The cycling parameters were: initial denaturation at 96°C for 3 min; 35 cycles of 96°C for 15 s, 52°C for 45 s, 72°C for 1 min 30 s; and final elongation at 72°C for 7 min. The same PCR primers were used for sequencing. Sequencing procedures used were as previously described (Abdel-Wahab & al. 2009, 2016). The nuclear LSU rDNA sequence of the new taxon was deposited at GenBank (FIG. 1). The sequence was aligned with *P. lignicola*, closely related genera in the *Halosphaeriaceae*, and representatives of *Microasaceae* and *Xylariales* using ClustalX (Thompson & al. 1997) and optimized manually. Maximum-parsimony (MP) and Maximum-likelihood (ML) phylogenetic analyses were carried out using PAUP* 4.0 (Swofford 2002). Maximum-likelihood (ML) analysis (Felsenstein 1981) was performed using heuristic searches with random stepwise addition of 100 replicates and TBR rearrangements. The best optimal model of nucleotide substitution for the ML analyses was determined using hierarchical likelihood ratio tests as implemented in Modeltest 3.7 (Posada & Crandall 1998). TrN was the best fit for LSU rDNA dataset. Bayesian phylogenetic analysis was performed in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) with the GTR model determined using MrModeltest 2.3 (Nylander 2004). Phylogenetic trees were visualized using NJplot (Perrière & Gouy 1996) and edited using Adobe Illustrator CS6.

Phylogenetic results

The LSU rDNA dataset comprised 47 sequences: 41 from *Halosphaeriaceae* and three from *Microasaceae*, with an outgroup of three from *Xylariales* (FIG. 1). The maximum parsimony dataset consisted of a total of 862

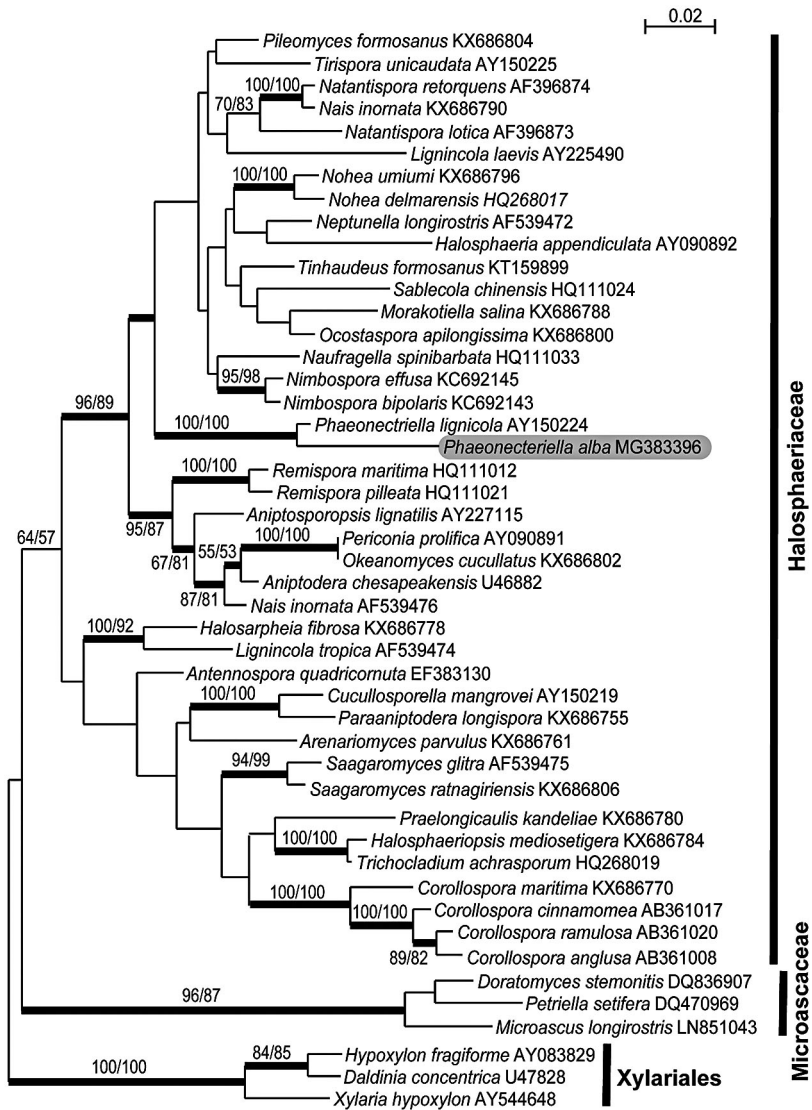


FIG. 1. Phylogenetic relationships of *Phaeoectriella alba* based on nucleotide sequences of the LSU rDNA along with the type species *P. lignicola* and other related genera in the *Halosphaeriaceae* and the closely related family *Microascaceae*. Representatives of the order *Xylariales* were used as outgroup. The maximum likelihood tree (ML) (–ln likelihood = 14607.49) was constructed as described in the text. Bootstrap support on the nodes represents ML and MP $\geq 50\%$. Branches receiving Bayesian PP $\geq 95\%$ are in bold. The sequence of the new species is in the grey box.

characters, of which 537 were constant, 83 variable and parsimony-uninformative, and 242 were counted as parsimony-informative. Maximum Parsimony analyses resulted in 18 most parsimonious trees, all of which had an equal tree length of 1105 steps, a consistency index of 0.4416, a retention index of 0.6672, and a rescaled consistency index of 0.2947. Maximum likelihood analysis yielded one tree ($-\ln$ likelihood = 14607.49; Fig. 1), and Bayesian analysis yielded trees with similar overall topology. The new species *P. alba* grouped consistently with the type species, *P. lignicola*, in all analyses with 100% statistical support in ML/MP and BYPP.

Taxonomy

Phaeonectriella alba Abdel-Wahab & Abdel-Aziz, sp. nov.

FIGS 2–13

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Differs from *Phaeonectriella lignicola* by its smaller, hyaline, conical, superficial ascomata, its shorter, wider asci, and its larger, symmetrical ascospores.

TYPE: Egypt, Aswan (24°05'42"N 32°53'06"E), in the River Nile, on decayed submerged culms of *Phragmites australis*, 3 January 2014, coll. Ahmed E. Abdel-Aziz (**Holotype**, AUMC-12004-H; GenBank MG383396).

ETYMOLOGY: Latin *alba* = white, the color of the ascomata.

Saprobic on decayed submerged stem of *Phragmites australis*.

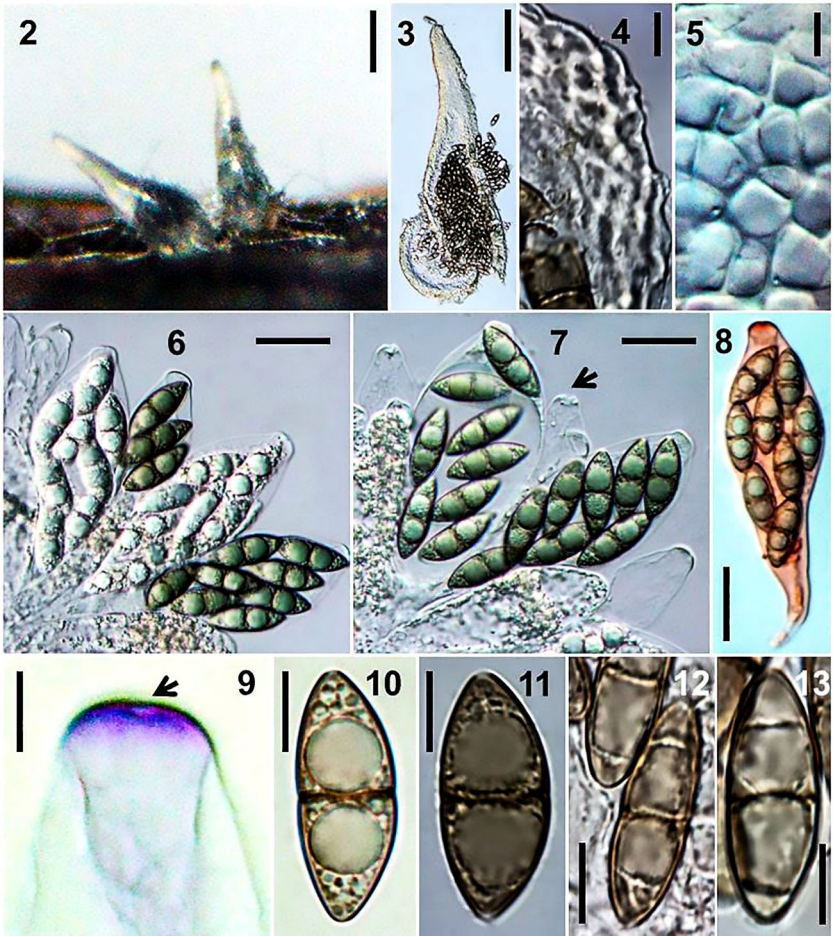
SEXUAL MORPH: ASCOMATA 200–325 μm long $\times$ 160–205 μm wide (mean = $251 \times 176 \mu\text{m}$, $n = 5$), conical, superficial, hyaline, membranous, solitary, ostiolate. NECK 175–225 μm long $\times$ 50–75 μm wide, periphysate, ostiolar canal $82.5 \times 12.5 \mu\text{m}$. PERIDIUM 12–27 μm thick, one-layered, hyaline, comprising 6–9 cell layers, thick-walled, forming a textura angularis. CATENOPHYSES early deliquescing. ASCI 77–138 $\times$ 28–41 μm (mean = $99 \times 34 \mu\text{m}$, $n = 30$), clavate, unitunicate, persistent, 4 or 8-spored, apically truncated, thickened, with apical simple pore and cytoplasm retraction below the apex. ASCOSPORES 25–33 $\times$ 8–13 μm (mean = $29 \times 11 \mu\text{m}$, $n = 50$), ellipsoid to broad ellipsoid, overlapping biseriate to multiseriate, hyaline when young, pale brown to brown at maturity, thin-walled, bi-celled, symmetrical, not constricted at the septum, with polar germ pores and without appendages.

ASEXUAL MORPH: Undetermined.

COMMENTS—TABLE 1 compares the morphology of *Phaeonectriella alba* and the other *Phaeonectriella* species. The type species *Phaeonectriella lignicola* is the most similar to *P. alba* but differs by its larger, hyaline to light-brown, globose to subglobose, immersed ascomata; its longer, narrower asci; and its smaller, longitudinally asymmetrical ascospores (Eaton & Jones 1970).

TABLE 1. Comparison of the morphology of *Phaeoneoctriella* species.

FUNGUS	ASCOMATA	NECK	CATENO- PHYSES	PERIDIUM	ASCI	ASCOSPORES
<i>P. alba</i> (this study)	200–325 × 160–205 µm, conical, superficial, hyaline	175–225 × 50–75 µm, periphysate	Early deliquescing	Forming a textura angularis	77–138 × 28–41 µm, 4- or 8-spored, apically truncate & thickened, with apical pore & cytoplasm retraction below the apex	25–33 × 8–13 µm, ellipsoid to broad ellipsoid, hyaline when young, (pale) brown when mature, symmetrical, not constricted at the septum, with polar germ pores, without appendages
<i>P. appendiculata</i> (Hyde & al. 1999)	150–300 µm diam, sub-globose, all to partly immersed, hyaline	No data	Not seen	Forming a textura angularis	100–140 × 24–30 µm, 8-spored, thin-walled, apically truncate & thickened, with apical pore & cytoplasm retraction below the apex	32–42 × 10–12 µm, fusiform, hyaline or pale brown, not constricted at the septum, with polar appendages
<i>P. lignicola</i> (Eaton & Jones 1970)	100–575 × 100–350 µm, globose to sub- globose, immersed, hyaline to pale brown	100–317 × 46–93 µm, periphysate	Not seen	Cells homogenous	100–417 × 22–35 µm, 8-spored, with apical pore	26–30 × 9.5–11 µm, ellipsoidal, hyaline when young, grey to brown when mature, asymmetrical, with polar germ pore in each cell
<i>P. lignicola</i> (Sensu Hyde & al. 1999)	100–575 µm diam, sub-globose, immersed, hyaline to pale brown,	No data	Not seen	Cells compressed	100–120 × 22–35 µm, 8-spored, apically truncate & thickened, with apical pore & cytoplasm retraction below the apex	26–30 × 9.5–11 µm, ellipsoidal-fusiform, hyaline or pale brown, grey-brown when mature, longitudinally asymmetrical, septum slightly constricted, with polar appendages



FIGS 2–13. *Phaeonectriella alba* (AUMC-12004-H, holotype): 2. Superficial ascomata on wood. 3. Vertical section through ascoma. 4. Vertical section through peridium showing thick-walled hyaline cells forming a textura angularis. 5. Surface view of peridial cells. 6–8. Asci at different stages of maturity (7. Apical simple pore (arrowed). 8. Ascus stained in Congo Red). 9. Apical part of ascus stained in Toluidine blue showing the simple pore (arrowed), truncate apical thickening and cytoplasmic retraction below apex. 10–13. Variously shaped ascospores. Scale bars: 2 = 140 μ m; 3 = 100 μ m; 4, 5, 9–13 = 10 μ m, 6–8 = 20 μ m.

Phaeonectriella appendiculata differs from *P. alba* by its globose to subglobose, immersed to erumpent ascomata; its smaller asci; and its larger ascospores with polar appendages (Hyde & al. 1999).

Ascospore appendages were not mentioned in the holotype of *P. lignicola* nor in the Taiwanese specimen (Eaton & Jones 1970, Jones 1995). However, the specimen from Mauritius identified as *P. lignicola* by Hyde & al. (1999) did produce polar appendages. This specimen should perhaps be designated as “*Phaeonectriella lignicola*” sensu Hyde & al. (1990), pending a molecular study to resolve whether or not polar appendages are typical of *P. lignicola*.

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